schools, and institutes named in her honour. Now, she will also lend her name to a research center, the CIB, part of the CSIC, the scientific research institution in which she spent her entire career. The **Centro de Investigaciones Biológicas Margarita Salas** proudly bears her name since November 21.

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# Spontaneous and Enzymatically Catalyzed Anomerization of Glucose 6-Phosphate and Anomeric Specificity of Related Enzymes*

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**Publication of Margarita Salas' first scientific discovery, obtained during her PhD in Alberto Sols' group at CIB**

## My thesis with Margarita Salas: between the CIB and the CBM

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It was so long ago that not even the bricks on the façade had started to fall. It was a spring morning in 1977 when I first climbed the stairs of the Centro de Investigaciones Biológicas at Velázquez 144. I had just completed a course in Molecular Genetics taught by Marta Rodríguez Inciarte at the Faculty of Biology of the Universidad Complutense de Madrid.

**The Center for Biological Research, at Velázquez 144.**

Marta was my mentor, and the person who convinced Margarita to let me join her laboratory. During her doctoral thesis, directed by Eladio Viñuela, Marta had generated the second ever-published complete restriction map of a viral genome, ordering the five restriction fragments that the *EcoRI* enzyme generated from the 18 kb of the *Phi29* genome! This had not been a trivial task, since 'the enzyme' (the sole restriction enzyme available at that moment) had to be purified through the use of fermenters that enabled the large-scale growth of the enzyme-producing bacterium. That's how I met José María Lázaro, the laboratory technician who oversaw this task, the best protein purifier I've ever met, and a faithful collaborator who worked with Margarita until his retirement a few years ago. As soon as I arrived at the laboratory, which I'll describe in detail shortly, Marta threw a lead apron on me and told me to keep my eyes open and soak up all the necessary know-how about phage purification with $^{32}$P-labelled DNA. Labelling was performed using 5 mCi in a single experiment. I opened my very pores, not just my eyes!

The laboratory was located in the "Viñuelas wing",

on the fourth floor of the old CIB, to the right as you emerged from the elevator, opposite the so-called "Davides" wing (after David Vázquez), also on the fourth floor, but with windows facing Calle Joaquín Costa. Located in front of the library of the Gregorio Marañón Institute and the office of Dr. Rodríguez Candela, director of the institute at that time, it consisted of three windows (the unit of measure of space in the former CIB), all facing Calle Velázquez, two corresponding to the laboratory and one to the office. The office was shared by Margarita and Eladio, and the laboratory space, where I also worked for a few months before we moved to the campus at the Autonomous University, was shared by Marta and Margarita.

If Marta was my "school master" in matters of incipient molecular biology, the person who taught me phage microbiology was the late-lamented Rafa Perez Mellado, who worked in the next laboratory of the same corridor. Even further away, at the end of the corridor, was the 'large' laboratory, where 10 years later I would meet two new arrivals to the CIB, the research fellows Santiago Rodríguez de Córdoba and Javier Paz-Ares, two of the brightest researchers ever to have passed through the CIB. The large laboratory housed a group of people who worked on virus morphogenesis, including Juan Antonio García Álvarez, who had begun his doctoral thesis one year before me and went on to become my inseparable phage companion.

**Two key features of the CIB** were lost after the move to the Centro de Biología Molecular: the "confession" and the "seminars/exams". The latter were held in a small room adjacent to the dining room of "Casa Visi" (which years later was incorporated into the dining room itself). These meetings consisted of conventional group seminars in which each researcher periodically presented his/her work. Eladio, who sat in the front row, would frequently interrupt the speaker and turn around to pose theoretical questions about the subject at hand to other researchers in the room. It goes without saying that these questions generated much uneasiness in the audience. The "confession", on the other hand, consisted of the summoning of a doctoral student to the office of Margarita and Eladio where, sitting between the two, the student would present an account of their experiments and conclusions. Margarita monitored daily the progress and planning of all researchers under her supervision, but Eladio also participated actively in the discussion of the results. The moment of the confession was feared, not because Margarita was at all aggressive with her students, but because it fully revealed the true extent of one's limitations and how much more there was to learn. The practice of "confession" ended when we moved at the end of that same year to the CBM, where Margarita and Eladio had independent offices. Nonetheless, Margarita continued to monitor my experiments daily, designing infinite controls that made the conclusions rock-solid, planning work on the calendar many days in advance, teaching me how to write the protocols so that the experiments could be reproduced by others at any time, and pointing me towards essential reading material.

For several years Juan Antonio and I were her only doctoral students. We enjoyed the luxury of her availability and we received our training through what today would be called "personalized attention" from a supervisor who, in addition to always treating us with almost maternal affection, was already a legend of molecular genetics.

In that room in Velázquez 144 I saw Margarita working for the first time at the bench. Before starting, she cleared her workplace and laid out on the wooden bench a mosaic of immaculately white filter papers that stood out against the black paint. Then, she took from her office some written protocols on which she had detailed all the stages of the procedure to be followed. After consulting the protocol, she placed on the filter papers all the material required: flasks, test tubes, conventional pipettes, micropipettes (which were then made of

glass and were reusable). Only when this ceremony had concluded would the experiment begin. It reminded me of an orchestra warming up before performing a complicated symphony, under the direction of a true master. I have never met a person who showed such a level of skill or concentration when executing complex scientific protocols.

**I would like to end with what for me is an unforgettable memory of Margarita** that sums up the extraordinary generosity with which she supported me on numerous occasions throughout my work. It was during the final stage of my doctoral thesis, towards the end of 1980, after we had transferred to the CBM. Ten years before, Juan Ortín had demonstrated (in the CIB) that phage DNA was covalently bound to a protein, and subsequent laboratory studies had identified this protein as the product of the *P3* gene. All available data pointed to that protein as an initiator of replication at the ends of *Phi29* DNA, but the definitive evidence was missing: synthesizing a complex consisting of p3 and dAMP that would serve as primer for the DNA polymerase at the ends of the DNA. After many unsuccessful efforts we managed to detect *in vitro*, through the incubation of protein extracts from phage-infected cells with dATP-α-[$^{32}$P], the formation of the p3-dAMP complex. While this was a promising start, much work remained to make the conclusions sufficiently solid for publication. During the following months we conducted a series of radiolabelling experiments in order to study all of the factors necessary for the reaction to occur. For each reaction, the protocol required prior gel filtration of the sample to remove the non-incorporated nucleotide before analysis by electrophoresis. Often I found myself with eight samples that needed to be urgently purified once the reaction was completed. Margarita realized right away that I simply couldn't handle so many samples, and offered to help me. Thus, the days that the radioactive nucleotide arrived I had to prepare two methacrylate screens and eight Sephadex columns packed in Pasteur pipettes. I knocked on the door of her office and Margarita came out, stood behind one of the screens and we passed the samples through, four per person, counting the drops that we collected in plastic tubes. I don't know how, but she always managed to finish before me. And she even had time to place the tubes in vials for the scintillation counter, so that I could rapidly analyse them. And so I was almost certainly the last person to work with Margarita Salas at the bench.

I recount this story because it is often noted, and rightly so, that one of Margarita's great achievements was creating an outstanding school. To do this it is necessary not only to have many (often very bright) students, but to also have a teacher that supports their students with generosity and unflagging dedication, qualities that she always exhibited.